Kleinere Mitteilungen.

(Biological Laboratory, University College, New York University).

A modification of CLEVELAND'S medium for *Entamoeba histolytica.*

By

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In the usual culture of *Entamoeba histolytica* there is a rapid change in p_H during the first 24 hours of incubation. For example, BOECK and DRBOHLAV (1925), who were the first to grow *E. histolytica* successfully, observed that the final p_H of their cultures varied with the medium used. Cultures in L. E. S. medium with an initial p_H of 7.4—7.7 showed the best growth and the least drop in p_H, while cultures with an initial p_H of 8.2 showed a drop to p_H 6.2 and poor growth of the amoebae. KOFOID and WAGENER (1925) likewise observed, in various types of media, a decided drop in p_H, the greatest observed being from 7.6 to 5.0. Since a p_H of 7.0 or higher is most favorable to growth of *E. histolytica*, any marked drop in p_H is detrimental to life of the culture. The writer, accordingly, attempted to prepare a buffered medium which would prevent such extensive p_H changes.

CLEVELAND'S (CLEVELAND and COLLIER, 1930) liver-infusion agar slants were prepared as follows:

DIFCO Bacto liver infusion agar .	30.0 gm.
Dibasic sodium phosphate .	3.0 „
Distilled water . .	1.0 liter

The medium was tubed, sterilized, and then slanted so as to avoid the formation of a butt. This type of slant is necessary for the reason that bacteria growing in the culture often produce gas; this causes the slant to rise in the tube and the amoebae drop beneath the slant.

Two buffer solutions were prepared in 0.85 % sodium chloride solution, one containing 9.47 gm of SÖRENSON's Na_2HPO_4 (anhydrous) per liter and the other 9.08 gm of SÖRENSON'S KH_2PO_4 per liter. A mixture of these two solutions, 82 parts of the sodium phosphate and 18 of the latter, was sterilized in the autoclave for 15 minutes at 15 pounds pressure, the final p_H being 7.4. Inactivated human serum was passed through a SEITZ filter into sterile flasks, tested for sterility, and then stored in a refrigerator until needed. This serum was diluted 1:10 with the sterile buffer mixture and then added, with sterile rice flour, to the sterile liver-infusion agar slants. The diluted serum showed a p_H of 7.4 ± 0.1, determinations being made with a TAYLOR slide colorimeter and LA MOTTE phenol red.

Tubes of medium were incubated to determine sterility, and on the following day were inoculated with *E. histolytica* (Cornell strain No. 370). Microscopic examinations were made after 24 hours and again after 48 hours in every case, and p_H determinations were made after 24 hours of incubation. After 24 hours, examination of wet preparations (4 mm objective and $10 \times$ ocular) showed 150 to 200 active amoebae in each field; the 48-hours cultures showed even heavier growth. This was in striking contrast to the results obtained previously with CLEVELAND's medium; during 7 months in which the strain had been carried in cultures in CLEVELAND's medium, the count ranged from 5 to 25 amoebae per 4 mm field. At the end of 24 hours, the p_H had dropped from 7.4 to 7.0, whereas the p_H of CLEVELAND's unmodified medium dropped from 8.6 to 6.6 in the same period.

The buffered medium and CLEVELAND'S medium were compared in cultures inoculated from the same source. Growth in the latter medium was invariably poor as compared with that in the buffered medium. Cross-inoculations were made from buffered cultures to unbuffered medium and from unbuffered cultures to buffered medium. The results were the same as before. This buffered medium with an initial p_H of 7.4 has been used for continued transfers of our strain of *E. histolytica* throughout a period of several months, and heavy growth has been obtained consistently. The results show that, for the Cornell strain No. 370, the present modification of

Cleveland's medium is far superior to the standard medium as formulated by Cleveland and Collier (1930). Our medium has also been used successfully in restoring to vigorous growth strains of *E. histolytica* which had almost died out in routine laboratory cultures.

Literature cited.

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